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Effects of weaning on concentrations of inhibin in follicular fluid and plasma of sows

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Summary. Changes in plasma and follicular fluid concentrations of inhibin were examined in sows after weaning at 28–32 days *post partum*. From 0 to 48 h after weaning, inhibin concentrations were 200–300 times higher in follicular fluid from small (<4 mm) and medium-large (≥ 4 mm) follicles than in ovarian venous plasma. Inhibin concentrations increased in follicular fluid from medium-large follicles at 24 and 48 h after weaning; concentrations in ovarian venous plasma were positively correlated with the number of medium-large follicles ($r=0.40$) and with ovarian venous plasma concentrations of oestradiol ($r=0.61$). Blood samples were collected for 30 days from sows ($n=6$) that exhibited oestrus within 5 days after weaning and from sows ($n=5$) that remained anoestrous for 11 days after weaning. Plasma inhibin concentrations rose in oestrous and anoestrous sows by 12 h and continued to rise for 60 h after weaning. Plasma inhibin concentrations rose further and were higher at 3.5–4.5 days after weaning in oestrous sows than in sows that remained anoestrous. After oestrus, plasma inhibin concentrations declined. At weaning, plasma concentrations of follicle-stimulating hormone (FSH) were higher in sows that subsequently exhibited oestrus than in sows that remained anoestrous. After weaning, plasma concentrations of FSH declined in both groups, reached a nadir at 2.5 days, and increased gradually in anoestrous sows; oestrous sows exhibited an FSH surge at oestrus. Plasma FSH returned to preweaning concentrations in both groups of sows at Days 7–8. The results demonstrated dynamic changes in plasma concentrations of FSH and inhibin in sows after weaning; an inverse relationship of these, with the exception during the preovulatory surge of FSH, typifies the porcine oestrous cycle.

Keywords: pigs (sows); ovarian function; oestrus; hormones

Introduction

Inhibin is a 32-kDa glycoprotein heterodimer secreted by the gonads and acting at the level of the pituitary, which inhibits secretion of follicle-stimulating hormone (FSH) (Ling *et al.*, 1985; Miyamoto *et al.*, 1985). In females, inhibin is secreted by the granulosa cells of the developing ovarian follicle and, in some species, by the corpus luteum (Henderson & Franchimont, 1981; Tsonis *et al.*, 1983; Rokukawa *et al.*, 1986; McLachlan *et al.*, 1987; Mann *et al.*, 1989). In addition to its function as an inhibitor of FSH release, inhibin secretion can serve as an index of granulosa

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cell function because its secretion is correlated with oestrogen secretion during rapid follicular development (Redmer *et al.*, 1986). Plasma concentrations of inhibin have been characterized during the oestrous cycle in several mammalian species, including pigs (Hasegawa *et al.*, 1988). The present study examined inhibin concentrations in porcine plasma and follicular fluid after lactational anoestrus.

The purpose was to investigate changes in the concentrations of inhibin in follicular fluid during early follicular growth (first 48 h after weaning) and to correlate these changes with concentrations of inhibin, oestradiol and progesterone in ovarian venous blood. A second study evaluated the temporal relationship of inhibin and FSH in peripheral plasma and compared sows that returned to oestrus without delay after weaning with sows that remained anoestrous.

Materials and Methods

Experiment 1. Forty-nine multiparous crossbred sows (28–32 days *post partum*) were randomly assigned to be unilaterally ovariectomized by previously described procedures (Martin *et al.*, 1986) at 0, 6, 12, 18, 24 and 48 h after weaning. One ovary was left intact, to identify sows that failed to return to oestrus promptly after weaning. After removal of litters, sows were checked once a day for signs of oestrus. Blood was collected by aspiration from a severed ovarian vein just before unilateral ovariectomy. Individual ovaries were chilled on ice and the numbers of small (<4 mm) and medium-large follicles (≥ 4 mm) were determined. Follicular fluid was collected and pooled within follicle-size classes; small (1–<4 mm), medium (4–6 mm) and large (>6 mm). Ovarian venous plasma and follicular fluid were stored at -20°C until assayed for concentrations of inhibin. Oestradiol-17 β and progesterone concentrations were determined in samples of ovarian venous plasma.

Experiment 2. Seventeen primiparous Duroc sows (28–32 days *post partum*) were surgically fitted with a jugular catheter 4–5 days before weaning (Ford & Maurer, 1978). This population of sows was selected for investigation because they have a high incidence of postweaning anoestrus. After weaning (Day 0), sows were monitored twice a day for signs of oestrus. Blood samples were collected via jugular catheters at 12-h intervals for 30 days after weaning from sows that exhibited oestrus and for 11 days from sows that remained anoestrous. More frequent blood samples (6-h intervals) were collected around the periods of expected oestrus. Blood samples were stored as plasma at -20°C and assayed for concentrations of inhibin, FSH and progesterone. Ovulation, or lack of it, was later confirmed by progesterone concentrations.

Detection of oestrus. Sows were housed in groups of three to six in Expt 1 and in individual gestation stalls in Expt 2. Sows were classified as in oestrus when, in direct contact with a mature boar, they exhibited the immobilization response (Signoret, 1970) after application of intermittent pressure to the lumbar region.

Radioimmunoassay. Plasma (400 μl) and follicular fluid (1 μl) concentrations of inhibin were determined according to methods described by Schanbacher (1988) for the radioimmunoassay of inhibin in ovine serum. The assay used rabbit antisera raised against the 30 N-terminal amino acids of the α chain of porcine inhibin, pI α (1–30) (Salk Institute, LaJolla, CA, USA). This peptide also served as standard and radiolabelled ligand. Concentrations of inhibin were expressed as moles of pI α (1–30)/ml plasma or follicular fluid. Addition of 50–400 μl of porcine plasma or 1–10 μl of porcine follicular fluid inhibited binding of ^{125}I -pI α (1–30) to the primary anti-sera (BDS-INH3) in a manner which was parallel to the standard curve. Intra- and interassay coefficients of variation averaged 7 and 18%, respectively. Although this antisera is directed against the α -subunit of inhibin, the immunoactivity that was determined is referred to as inhibin. Follicular fluid and plasma may contain some inhibin α -subunit in addition to the intact α , β -dimer, but limited data indicate that the concentration of α -subunit is low in porcine follicular fluid (Knight *et al.*, 1989). Michel *et al.* (1989) reported that an antiserum against amino acids 1–32 of porcine α -subunit detected only one immunoreactive fraction of medium from cultured porcine granulosa cells.

Plasma concentrations of FSH were determined by a heterologous radioimmunoassay using rabbit anti-ovine FSH antisera (JAD 17-679; Krystek *et al.*, 1985), ^{125}I -LER-1976-A2 and USDA pFSH-B1 as the reference standard. Intra- and interassay coefficients of variation averaged 13 and 6%, respectively. Inhibition of binding of the iodinated ovine FSH by increasing volumes of porcine serum paralleled that of the porcine reference standard. Little cross-reactivity occurred with porcine luteinizing hormone (LH; USDA-pLH; binding of ^{125}I -FSH was 100, 94, 88 and 83% in presence of 1, 10, 100 and 1000 ng, respectively).

Concentrations of oestradiol and progesterone were determined by radioimmunoassays described by Christenson *et al.* (1985). Before assay, progesterone was extracted from plasma with heptane, and oestradiol was extracted from plasma with ethyl ether. Follicular fluid was diluted with phosphate-buffered saline and assayed directly.

Statistical analysis. The data were analysed by the General Linear Model (GLM) least-squares analysis-of-variance procedure of SAS (1985). If the analysis of variance revealed a significant F-statistic, treatment means were separated by the predicted difference option of GLM. Data are presented as means \pm s.e.m.

In Expt 1, the effect of time after weaning on ovarian venous plasma concentrations of inhibin, oestradiol and progesterone was examined by one-way analysis of variance. Concentrations of inhibin in follicular fluid after weaning were examined by split-plot analysis of variance. The variation associated with gilt nested within time after weaning was used to test for a significant effect of time after weaning. Similarly, the source of variation associated with the interaction [gilt (time) \times follicle size] was used to test the significance of the interaction of time after weaning and follicle size. The effect of follicle size upon concentrations of inhibin in follicular fluid was tested with error residual.

In Expt 2, the first analysis used a split-plot analysis of variance to examine concentrations of inhibin and FSH in plasma samples collected from gilts experiencing oestrus after weaning and from gilts remaining anoestrous for up to 11 days after weaning. The effect of oestrous classification on concentrations of inhibin and FSH was tested with the source of variation associated with gilt nested within oestrous class. The second analysis examined plasma concentrations of inhibin and FSH for 30 days after weaning in the six gilts which experienced oestrus. Time after weaning and gilt were included as sources of variation in the model. After these sources were removed, the residual correlation between inhibin and FSH was obtained with the MANOVA/PRINT option of GLM.

Results

Experiment 1

Forty-two of the 49 sows exhibited oestrus within 8 days after weaning with a mean interval to oestrus of 5.2 ± 0.15 days. Return to oestrus was similar for all six groups of unilaterally ovariectomized sows. Inhibin concentrations in ovarian venous plasma (Table 1) increased from 53 fmol/ml at weaning to 95 fmol/ml 12 h after weaning ($P < 0.10$). After this initial increase, inhibin decreased to a nadir of 46 fmol/ml at 24 h, which was again followed by an increase to 101 fmol/ml by 48 h after weaning. Ovarian venous plasma concentrations of oestradiol and progesterone changed in a similar pattern, oestradiol being greater at 6 h than at 0 h and progesterone being greater at 48 h. Concentrations of inhibin in ovarian venous plasma were positively correlated with concentrations of oestradiol ($r = 0.61$, $P < 0.001$) and with the number of medium-large follicles present on the ovary ($r = 0.40$, $P < 0.01$), but not with ovarian venous concentrations of progesterone.

Concentrations of inhibin in follicular fluid were higher than those in ovarian venous plasma. Medium-large follicles had higher ($P < 0.01$) concentrations than small follicles, 18.7 ± 0.3 vs. 16.8 ± 0.4 pmol/ml; due primarily to an increase ($P < 0.05$) in concentrations of inhibin in medium-large, but not in small, follicles at 24 and 48 h after weaning (Fig. 1).

Table 1. Concentrations of inhibin, oestradiol, and progesterone in ovarian venous plasma of sows 0–48 h after weaning

	Time after weaning (h)						s.e.
	0	6	12	18	24	48	
<i>n</i>	7	6	6	8	8	7	—
Inhibin (fmol/ml)	53	57	95	62	46	101 ^a	12
Oestradiol (ng/ml)	0.1	0.3 ^a	0.3 ^a	0.2	0.2	0.5 ^a	0.1
Progesterone (ng/ml)	1.6	2.5	3.3	1.8	1.6	4.1 ^a	0.8

^aDifferent from concentration at time 0 ($P < 0.05$).

Experiment 2

Nine of the 17 sows returned to oestrus within 6 days after weaning. The six oestrous sows that were selected at random for determination of blood hormone profiles weaned 6.8 ± 0.8 piglets, and the five anoestrous sows weaned 7.0 ± 1.3 piglets. Three sows were excluded from the study because the catheter of one failed, one showed oestrus on Day 11, and one did not show oestrus, but had raised concentrations of plasma progesterone. All sows classified as anoestrous had < 0.25 ng progesterone/ml plasma during the 11 days after weaning. The six oestrous sows had

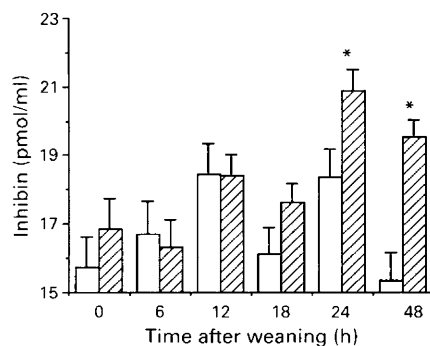


Fig. 1. Concentrations of inhibin in porcine follicular fluid of small (1–4 mm, □) and medium to large (≥4 mm, ▨) follicles during the first 48 h after weaning; *greater than at hour 0, $P < 0.05$.

an interval of 4.7 ± 0.2 days from weaning to oestrus and 20.8 ± 0.3 days between first and second oestrus. Their plasma progesterone concentrations reached 20 ± 0.7 ng/ml on Day 15 after weaning (Day 10.8 ± 0.2 of the oestrous cycle).

Concentrations of inhibin in plasma increased in oestrous and anoestrous sows during the first 60 h after weaning (Fig. 2). Average concentrations of inhibin had increased in both groups ($P < 0.05$) by 12 h, compared with 0 h. Inhibin concentrations continued to rise in oestrous sows, reaching peak concentrations near the time of oestrus. In contrast, plasma inhibin concentrations declined in sows that remained anoestrous, reaching a nadir at 4.5 days. As a result, plasma inhibin concentrations were greater ($P < 0.01$) 3.5–4.5 days after weaning in sows experiencing oestrus than in sows remaining anoestrous. Concentrations of plasma inhibin were again similar in both groups by 5.5–6 days and remained high until Day 10 in both groups compared with concentrations at weaning.

At weaning, sows that subsequently experienced oestrus had higher ($P < 0.05$) concentrations of plasma FSH than anoestrous sows (Fig. 2). Plasma FSH declined ($P < 0.01$) in both groups after weaning, average concentrations reaching a nadir at 2.5 days. FSH concentrations remained low in oestrous sows until Day 4, peaked at the time of oestrus, fell to a second nadir at 5.5 days and then increased from Day 6 to Day 8. FSH concentrations in anoestrous sows increased steadily from 2.5 to 6 days. By 8 days after weaning, plasma concentrations of FSH in both groups were again similar to those observed at weaning.

During the two follicular phases of the 30-day postweaning period, plasma inhibin concentrations increased, while concentrations of FSH decreased until the time of the oestrous surge of FSH (Fig. 3). After oestrus, inhibin concentrations decreased as concentrations of FSH increased, and remained high throughout the luteal phase. Overall, there was a negative residual correlation ($r = -0.24$, $P < 0.001$) between plasma concentrations of FSH and inhibin throughout the oestrous cycle. Mean plasma progesterone concentrations exceeded 1 ng/ml from Day 8 to Day 22 and from Day 27 to Day 30 after weaning.

When data for the second postweaning oestrus were replotted and aligned with the preovulatory release of FSH (Fig. 4), patterns of change in inhibin and FSH concentrations were similar to those observed at first postweaning oestrus (Fig. 2). Peak concentration of inhibin was 1.5 times greater during second postweaning oestrus than during the first. The abrupt decrease in mean inhibin concentrations on Day -1 reflects unexplained low concentrations in two of the six sows. Second postweaning oestrus occurred at Day -0.8 ± 0.2 relative to the FSH peak. Plasma progesterone concentrations were 11.2 ± 1.2 ng/ml on Day -5, decreased to 0.8 ± 0.1 on Day -3, remained below 0.5 ng/ml from Day -1 to Day 1 and increased to 1.8 ± 0.2 and 4.9 ± 0.5 ng/ml on Days 2 and 3 after the preovulatory release of FSH.

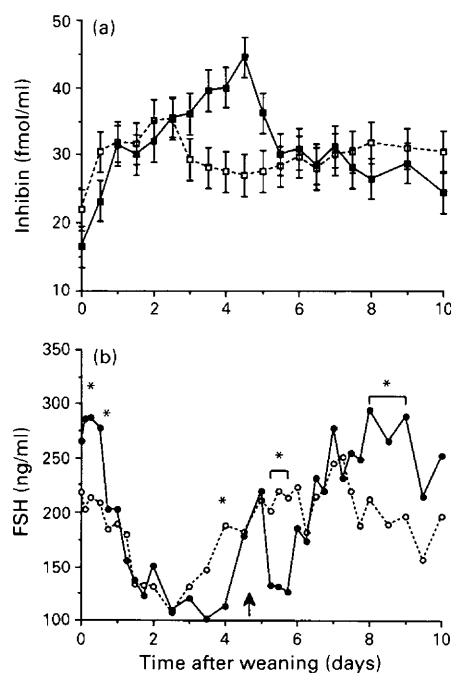


Fig. 2. Concentrations of (a) plasma inhibin and (b) porcine follicle-stimulating hormone (FSH) in oestrous (—) and anoestrous (---) sows during the first 10 days after weaning. Arrow indicates initiation of oestrus in oestrous sows; *oestrous sows differed from anoestrous sows, $P < 0.05$; s.e.m. was 20.8 ng FSH/ml.

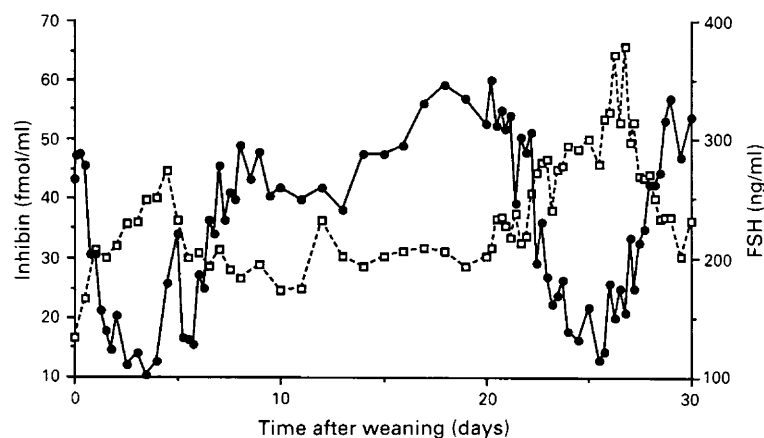


Fig. 3. Concentrations of plasma inhibin (□) and porcine follicle-stimulating hormone (FSH, ●) in oestrous sows during the first 30 days after weaning; s.e.m. was 5.3 fmol inhibin/ml and 27 ng FSH/ml.

Discussion

The study shows that rapid changes in serum FSH and inhibin occur after weaning in sows. The inverse relationship between these hormones during postweaning folliculogenesis is similar to that

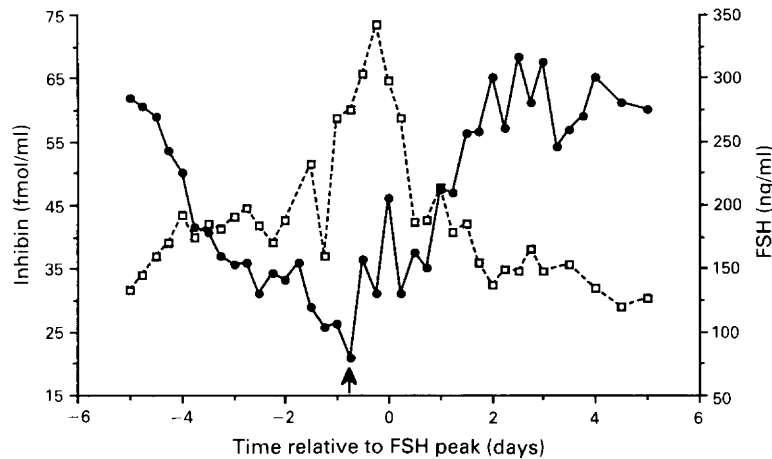


Fig. 4. Concentrations of plasma inhibin (□) and porcine follicle-stimulating hormone (FSH, ●) during second postweaning oestrus; s.e.m. was 4.4 fmol inhibin/ml and 23 ng FSH/ml. Arrow indicates initiation of oestrus.

reported by Hasegawa *et al.* (1988) during the oestrous cycle of gilts. Inhibin concentrations increased by 12 h after weaning in ovarian venous and peripheral plasma, after which plasma FSH concentrations decreased. The exception to the inverse relation between these hormones was during the preovulatory increase in FSH and subsequent decrease in both of these hormones before ovulation. Stevenson *et al.* (1981) proposed that FSH secretion in sows was modulated by an ovarian factor other than oestradiol, and Hasegawa *et al.* (1988) identified inhibin as the major regulator of FSH secretion during the oestrous cycle. They observed that increased inhibin secretion, followed by decreased FSH secretion, occurred before oestradiol increased during follicular development.

Ovarian venous plasma concentrations of inhibin and oestradiol are positively correlated during periods of rapid follicular growth (Redmer *et al.*, 1986; Table 1); thus, their synthesis and secretion are probably coupled. Indeed, plasma inhibin concentrations increased through oestrus in sows that ovulated (Fig. 2). After weaning, the number of small follicles decreases and the number of medium and large follicles increases (Dyck, 1983). In conjunction with growth of medium and large follicles, their follicular fluid concentrations of inhibin increase (Fig. 1). These observations, coupled with the positive correlation of follicular size with mRNAs for α - and β_A -subunits of inhibin (Guthrie *et al.*, 1991), indicate that growing follicles are probably the primary source of circulating inhibin.

It was observed that sows with higher plasma FSH concentrations at weaning were more likely to exhibit oestrus and ovulate earlier than sows with lower FSH concentrations. Sows that remain anoestrus after weaning initiate follicular development as shown by increased oestradiol (Armstrong *et al.*, 1986) and inhibin secretion in association with reduced FSH secretion (Fig. 2). Follicular growth in these sows does not continue through preovulatory development; thus follicles may not receive sufficient LH stimulation because secretion of LH in anoestrous sows is more sensitive to the negative feedback effects of oestradiol (Almond & Dial, 1990). Ovarian follicles of anoestrous sows remain responsive as ovulations are induced by exogenous gonadotrophins or LH-releasing hormone (LHRH) (Britt *et al.*, 1985).

The primary difference between observations in the present study and those of Hasegawa *et al.* (1988) is the preovulatory increase in FSH secretion that was observed at both first and second oestrus after weaning. Guthrie & Bolt (1985), Cox & Britt (1986), Cox *et al.* (1987), Kelly *et al.* (1988) and Mukai *et al.* (1989) reported FSH profiles similar to those in our study; a distinct preovulatory rise and fall in FSH coincident with the preovulatory release of LH, followed by a postovulatory increase in FSH. In contrast, in the reports by Stevenson *et al.* (1981), van der Wiel

et al. (1981) and Hasegawa *et al.* (1988), a preovulatory increase in FSH secretion coincident with increased LH was not obvious. Assuming that pigs have a preovulatory release of FSH, regulation of FSH secretion appears similar to that in rats (Rivier *et al.*, 1989). LHRH stimulates the immediate preovulatory increase in FSH and, after the ovulatory release of gonadotrophins, inhibin secretion abruptly declines reflecting differentiation of granulosa cells. After ovulation, FSH increases because inhibin secretion is low. Secretion of FSH is high during the luteal phase of the oestrous cycle in pigs, but the magnitude of this increase varies among reported studies: 1.5–2 times higher than the preovulatory nadir in some studies (van der Wiel *et al.*, 1981; Hasegawa *et al.*, 1988) compared with an increase of 3–4 times in others (Mukai *et al.*, 1989; Fig. 3). Determination of the true profile of FSH secretion in sows awaits bioassay data.

In conclusion, the observed changes in plasma inhibin and FSH concentrations in sows showed that secretion of these hormones is a reciprocal relationship, except during the preovulatory release of gonadotrophins. Secretion of these two hormones changes rapidly after weaning in sows that ovulate within 1 week of weaning and in those that become anoestrous.

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